UVB Irradiation Increases the Release of SCF from Human Epidermal Cells

To the Editor:

Stem cell factor (SCF) is a multifunctional growth factor that is critical for the survival and maturation of melanocytes, and which also affects dermal mast cell growth, accumulation and degranulation. It is one of the membrane-anchored growth factors that are processed to soluble forms by proteolytic cleavage, and which function both as soluble and as membrane molecules (Ashman, 1999). Recently, Hachiya *et al* (2001) reported that ultraviolet B (UVB) irradiation increases membrane-bound SCF content in human epidermis and in cultured keratinocytes, and they suggested the participation of this type of SCF in UVB-induced melanogenesis, in which this molecule would bind to the c-kit receptor on melanocytes, resulting in persistent c-kit activation. That study however, did not fully examine the relationship of soluble type SCF, as they could not detect it. On the other hand, one report showed that chronic UVB exposure increases the number of dermal mast cells, accompanied by increased epidermal SCF expression in albino hairless mice (Kligman and Murphy, 1996), whereas another study showed that subcutaneous injection of recombinant SCF or transgene expression to produce soluble SCF in the epidermis causes cutaneous mast cell hyperplasia and cutaneous hyperpigmentation (Grichnik *et al*, 1995; Kunisada *et al*, 1998). These observations led us to speculate that epidermal SCF also acts as a soluble factor in UV-exposed skin. Thus, in this study, we examined the possibility that stimulation of SCF release occurs in UVB-exposed cultured keratinocytes and in human epidermis.

Normal human epidermal keratinocytes were purchased from Kurabo (Osaka, Japan) and were cultured in MCDB153HAA medium (Kyokuto, Tokyo, Japan) supplemented with ethanolamine (0.1 mM), phosphoethanolamine (0.1 mM), hydrocortisone (0.5 nM), epidermal growth factor (0.1 ng per mL, Sigma, St Louis, Missouri), insulin (5 μg per mL, Sigma), and bovine pituitary extract (BPE; 4 μL per mL, Kurabo). At sub-confluence, the cells were transferred to starvation medium (without BPE) 24 h prior to UVB stimulation, which was carried out twice at a 24-h interval using FL20SE lamps (Toshiba, Tokyo, Japan). The culture media were harvested 24 h after the second UVB irradiation and were analyzed using an ELISA. As shown in Fig 1*a*, the amount of SCF in the culture medium was increased by UVB stimulation in a dose-dependent manner up to 20 mJ per cm^2, and then decreased at a dose of 40 mJ per cm^2 with an accompanying reduction of cell viability. Further, western blot analysis revealed that UVB irradiation caused an increase of the 31-kDa SCF protein band, which peaked at a dose of 20 mJ per cm^2 (Fig 1*b*). In addition, the molecular weight of that band corresponded to the cleaved form of full-length membrane-bound SCF. These results show that UVB irradiation at non-cytotoxic doses increases the release of SCF by cultured human keratinocytes. Our *in vitro* findings were further supported by an experiment using a living skin equivalent (MEL-300, Kurabo), in which UVB stimulation also resulted in increased SCF content in the culture medium (data not shown). In a previous study, performed under conditions that induced a significant reduction of cell viability by UVB irradiation, a UVB-induced upregulation of SCF release could not be detected (Hachiya

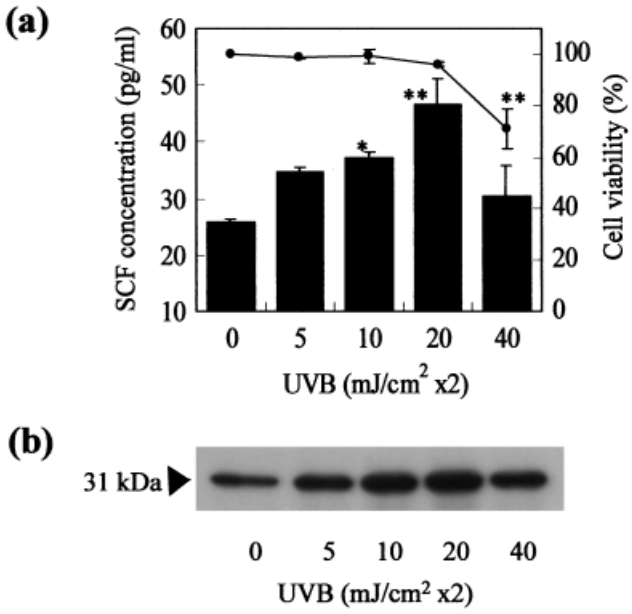

Figure 1
UVB stimulation increases SCF release from cultured human keratinocytes. (*a*) Effects of UVB irradiation on SCF release and cell viability of keratinocytes. The bars and polygonal line show the SCF content in culture medium and cell viability, respectively. SCF content was determined using an ELISA (Quantikine, R&D Systems, Minneapolis, Minnesota). Cell viability was measured using an alamarBlue Assay (Alamar Biosciences, Sacramento, California) 24 h after the second UVB irradiation. Each value is presented as the mean ± SD of three determinations. $^{**}p<0.01$, $^{*}p<0.05$ as compared with the control by Dunnett's test. (*b*) Detection of soluble SCF by western blotting. Samples were concentrated 10-fold using a Centricon YM-10 (Millipore, Bedford, Massachusetts) and were separated by gel electrophoresis. Rabbit anti-SCF (Immuno-Biological Laboratories, Gunma, Japan) was used as a primary antibody.

Abbreviations: SCF, stem cell factor; UP, urticaria pigmentosa; UV, ultraviolet

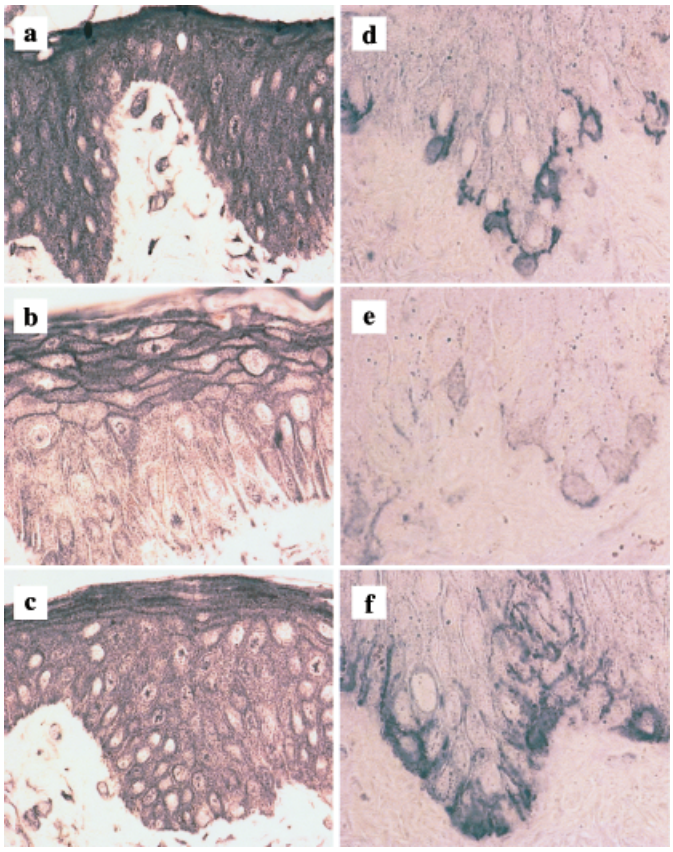

Figure 2
Changes of immunoreactivity toward SCF/c-kit in human epidermis following UVB exposure. Immunostaining for SCF (*a–c*) and c-kit (*d–f*) at pre-exposure (*a*, *d*), and 3 (*b*, *e*) and 10 days (*c*, *f*) after exposure. Acetone fixed-cryostat 5-μm sections were incubated with polyclonal rabbit anti-SCF (Santa Cruz Biotechnology, California) or a polyclonal rabbit anti-c-kit (DAKO, Carpinteria, California). Immunolocalization of the primary antibody was performed using the avidin–biotin peroxidase complex method (ABC-Elite kit, Vector Laboratories, Burlingame, California), with nickel-diaminobenzidine as the substrate.

et al, 2001). Therefore, it seems that responses induced by UVB irradiation at non-cytotoxic levels were not considered in that study.

Following approval from the Ethical Committee of Kanebo Basic Research Laboratory, we also administered UVB irradiation, which was performed with 2 MED of UVB (0.09–0.12 J per cm^2; FL20SE lamps), to the upper-back area (2.0 cm $\times$ 2.0 cm) of 10 healthy male Japanese volunteers who gave their written informed consent, after which analyses of SCF and c-kit expression were performed 3 and 10 days following the irradiation on skin biopsy specimens. Five millimeter-punch biopsies were taken from each exposed region following the experimental irradiation. As a control, non-exposed skin areas were also biopsied from all subjects. SCF was detected by immunohistochemistry throughout the epidermis in intact skin, as previously reported (Fig 2*a*: Longley *et al*, 1993; Weiss *et al*, 1995). Three days after the irradiation, cytoplasmic staining of SCF was found to be reduced in epidermal keratinocytes, whereas membrane-associated SCF staining was clearly seen in the epidermal upper spinous and granular layers (Fig 2*b*). Thereafter, those levels returned to nearly normal by day 10 (Fig 2*c*). Further, a decreased expression of c-kit on melanocytes was observed on day 3 after UVB (Fig 2*d–f*).

Changes in epidermal SCF-immunostaining patterns to reduced cytoplasmic staining and membrane-associated staining have been reported in lesional urticaria pigmentosa (UP), a skin disease characterized by dermal accumulations of mast cells and increased epidermal melanin. Such changes are considered to result from the altered metabolism of SCF, which induces an elevation of soluble SCF in the skin (Longley *et al*, 1993; Weiss *et al*, 1995). On the other hand, it has been reported that the c-kit receptor is internalized and degraded after binding its soluble ligands (Shimizu *et al*, 1996), and our experiment showed decreased c-kit expression on day 3 (Fig 2*e*). Therefore, altered SCF immunoreactivity observed on day 3 might be the consequence of enhanced SCF liberation in the epidermis.

From reports that serum SCF levels are elevated in patients with various skin diseases (e.g. atopic dermatitis, scleroderma) and that those levels recover with clinical improvement (Kanbe *et al*, 2001; Yamamoto *et al*, 2001), there is every probability that SCF shedding occurs in cutaneous tissues. Furthermore, it has been revealed that several proteases are involved in the release of SCF (Pandiella *et al*, 1992; Gallea-Robache *et al*, 1997; Longley *et al*, 1997; Heissig *et al*, 2002), and the profiles of some of these enzymes in the skin are altered by UV exposure (Fisher *et al*, 1997). Thus, it seems that UV irradiation of the skin establishes a condition in which SCF shedding occurs.

In conclusion, SCF release from cultured epidermal cells was increased by UVB radiation, which also altered the SCF/c-kit expression pattern in human epidermis. These results suggest that SCF/c-kit signaling in UV-exposed skin occurs through soluble SCF, as well as through the membrane-bound form.

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Sun Protection, Vitamin D Deficiency, and Management of Cutaneous Oncology in Organ Transplant Recipients (OTR)

To the Editor:

We have read with great interest the excellent article of Carucci (2004) who has carried out a comprehensive review of epidemiology, pathogenesis, and clinical outcome of skin cancer in organ transplant recipients (OTR) and has given recommendations for its medical management. We would like to add to this discussion an important topic that has not been addressed by John A. Carucci, but that we dermatologists must be aware of: the fact that strict sun protection may lead in OTR to the severe health risk of vitamin D deficiency. We know that OTR may develop 25-hydroxyvitamin D deficiency (Segal *et al*, 2001), likely at least in part due to lack of sun exposure. But an epidemiologic association between vitamin D deficiency and a broad variety of severe health problems including various types of malignancies (e.g. colon-, prostate-, and breast cancer) has now been reported convincingly (Garland *et al*, 1989; Grant, 2002). It is now evident that a broad variety of tissues including skin, prostate, colon, and breast express the enzyme (25-hydroxyvitamin D-1α-hydroxylase) to convert 25-hydroxyvitamin D to its active form, 1,25-dihydroxyvitamin D (Schwartz *et al*, 1998; Tangpricha *et al*, 2001). 1,25-dihydroxyvitamin D is now not exclusively considered as a calciotropic hormone but also as a locally produced regulator of different cellular functions including cellular growth (Schwartz *et al*, 1998; Tangpricha *et al*, 2001). As John Carucci points out, regular dermatological follow-up must be provided for OTR. In this context, we would like to strengthen the fact that it is of high importance to detect and to treat 25-hydroxyvitamin D deficiency in OTR. Recommendations for the oral treatment of vitamin D deficiency have been reported previously (Malabanan *et al*, 1998; Vieth, 1999). A single dose of 50,000 IU vitamin D once a week for 8 wk is efficient and safe. Another means of guaranteeing vitamin D sufficiency is to give 50,000 IU of vitamin D once a month. We would like to accentuate the fact that careful monitoring of vitamin D status and oral substitution in case of vitamin D deficiency is of high importance for OTR, as we have recommended previously (Reichrath, 2003, 2004). This will protect OTR sufficiently against the serious health problems of 25-hydroxyvitamin D deficiency without further increasing their risk of developing squamous cell carcinoma of the skin or other types of UV-induced skin cancer.

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Abbreviation: OTR, organ transplant recipients

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